

# Spinal Pharmacology of Thermal Hyperesthesia Induced by Incomplete Ligation of Sciatic Nerve

## I. Opioid and Nonopioid Receptors

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Mechanisms underlying the pain state in humans that follows incomplete injury to peripheral nerve are little understood. To gain better understanding of this phenomenon, this study evaluated the effects on the thermally evoked hind-paw withdrawal latency produced by the intrathecal administration of morphine, U-50 488H (U-50), (D-Pen2, D-Pen5)-enkephalin (DPDPE), ST-91, baclofen, muscimol, and 5'-N-ethylcarboxamide-adenosine (NECA) in normal rats and in rats with a hind paw rendered unilaterally hyperesthetic by the unilateral application of loose ligatures to the sciatic nerve. In the animals with one ligated nerve, the hind-paw latency for the ligated paw was typically 2-4 s less than that for the nonligated paw, at 7-11 days postoperatively. In normal rats prepared with chronic intrathecal catheters, dose-dependent increases in paw withdrawal latency were observed; the order of activity was: baclofen, ST-91, morphine, muscimol, DPDPE >> U50, NECA  $\geq$  0. In the nonligated (nonhyperesthetic) paw of the lesioned animals, intrathecal agents also resulted in a dose-dependent increase in the paw withdrawal latency; the order of potency was: NECA, baclofen, morphine, ST-91, muscimol, DPDPE > U50  $\geq$  0. For both NECA and morphine, the median effective dose (ED<sub>50</sub>) values were significantly less in the nonhyperesthetic hind paw. For the hyperesthetic paw, the dose-response curves were parallel to those obtained concurrently in the nonhyperesthetic paw but were shifted significantly to the right by a factor of 3-5, with the rank order of activity in the hyperesthetic paw being baclofen, morphine, muscimol, DPDPE > ST-91, NECA, U50  $\geq$  0. These data indicate that 1) spinal receptor systems that alter thermal afferent processing in the normal animal are similarly active in the hyperesthetic paw of the lesioned animal; and 2) unexpectedly, despite similar predrug response latencies, certain receptor systems regulating the response in the nonhyperesthetic paw of the lesioned rat (morphine and NECA) show greater activity than in the nonlesioned rat. (Key words: Analgesia. Nerve, injury: hyperesthesia. Receptors: Adenosine; GABA-B; opioid;  $\alpha$ -adrenergic.)

IN HUMANS, incomplete injury to the peripheral nerve can give rise to apparently spontaneous burning pain sensations. An additional component of this syndrome is the development of a thermal or mechanical hyperesthesia.<sup>1</sup> The mechanisms underlying these pain states are little understood, although the association of the syndrome with nerve injury has led to the designation of these causalgic

syndromes as neurogenic in origin. Of particular interest is the clinical report that such causalgic pain appears to be relatively refractory to standard analgesics such as opioids.<sup>2</sup> Such observations, though controversial,<sup>3</sup> suggest either that the pain state represents the excessive activation of an otherwise sensitive circuit (thereby requiring more receptor occupancy) or that the pain state itself reflects a system which, unlike that activated by the acute stimulation of C fibers, is not coupled with opioid receptors.

An important difficulty in defining this clinical state has been the absence of applicable animal models with correlative validity. Recently, it was found that application of multiple loose ligatures around the sciatic nerve resulted in a prominent demyelination and degeneration of large fibers with a preservation of small afferent nerves. After an interval of 3-5 days, the lesion will render the paw thermally hyperesthetic.<sup>4</sup> We have initiated a series of studies to characterize systematically the spinal pharmacology of this hyperesthesia by the intrathecal administration of receptor-specific agonists into animals with unilateral sciatic nerve constriction.

## Materials and Methods

The following investigations were carried out under a protocol approved by the Institutional Animal Care Committee, University of California, San Diego.

## ANIMAL PREPARATION

### *Intrathecal Catheter*

Male Sprague-Dawley rats (250-300 g) were prepared with chronic catheters in the lumbar subarachnoid space.<sup>5</sup> Briefly, under halothane anesthesia, a PE-10 catheter was advanced through an incision in the atlantooccipital membrane to a position 9 cm caudal to the cisterna at the level of the lumbar enlargement. The catheter was externalized on the top of the skull and sealed with a piece of steel wire. The wound was closed with 3-0 silk sutures. Rats showing neurologic deficits postoperatively were discarded.

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*Sciatic Nerve Ligation*<sup>4</sup>

After intrathecal implantation, the hyperesthetic state was induced by incomplete ligation of the sciatic nerve. Anesthesia was induced with halothane 4% and maintained at a concentration of 2–3% as needed. After a skin incision, the biceps femoralis of each leg was bluntly dissected at mid thigh to expose the sciatic nerves. Each nerve was then carefully mobilized, with care taken to avoid undue stretching. At this time, four 4-0 chromic gut sutures each were tied loosely with a square knot around the right sciatic nerve. We used a small and brief twitch in the muscle surrounding the exposure as an indicator of the desired degree of constriction. The left sciatic nerve was only mobilized. Both incisions were closed layer to layer with 3-0 silk sutures, and the rats were allowed to recover from anesthetics.

After sciatic nerve ligation, the animals were maintained individually in clear plastic cages with solid floors covered with 3–6 cm sawdust. Animals appropriately prepared would show a mild dorsal rotation of the ligated paw and a mild to moderate degree of foot drop. All animals postoperatively displayed normal feeding and drinking.

## NOCICEPTIVE THRESHOLD

The thermal nociceptive threshold was measured with a device similar to that previously reported.<sup>6</sup> The rats were placed in a clear plastic cage (10 cm/20 cm) placed on an elevated floor of clear glass (2 mm thick). A radiant heat source (halogen projector lamp CXL/CXP 50W 8V, Ushio, Tokyo, Japan) was contained in a movable holder placed beneath the glass floor. The radiant heat source's aperture was 4 mm in diameter. The voltage to the thermal source was controlled by a constant voltage supply. To reduce the variability in plate surface caused by room temperature, the interior of the box under the animal was prepared with a heat source such that the under-plate temperature was regulated to 30° C. The calibration of the thermal test system was such that the average response latency in normal untreated rats, measured prior to the initiation of an experimental series, was 10 ± 0.5 s.

To initiate a test, the rat was placed in the box and allowed 5–10 min to adjust to it. The under-floor heat source was then positioned such that it focused at the plantar surface of one hind paw where it was in contact with the glass. Care was taken not to focus the light source on the skin that was off of the glass plate. The light was then activated; activation of the light initiated a timing circuit. The time interval between the application of the light beam and the brisk hind-paw withdrawal response was measured to the nearest 0.1 s. Cut-off time in the absence of a response was 20 s. This value was then assigned as its response latency.

## GENERAL BEHAVIOR

Motor function was evaluated at each time point during the dose–response study by the observation of two specific behaviors, as follows. 1) Placing/stepping reflex: this response was evoked by drawing the dorsum of either hind paw across the edge of the table. This stimulus elicits an upward lifting of the paw from the surface of the table (stepping). 2) Righting reflex: a rat placed horizontally with its back on the table will normally show a immediate coordinated twisting of the body around its longitudinal axis to regain its normal posture.

## EXPERIMENTAL PROTOCOLS

In these experiments, the effects of intrathecally administered agents on the escape latency was assessed in normal (nonligated) rat and in the normal (left nerve nonligated) and hyperesthetic (right sciatic nerve ligated) paws of operated rats.

*Normal Rat Study*

Before drug injection, three latency measurements were taken for each hind paw with 5 min intervals between consecutive tests as the baseline data. After the drug injection, one latency measurement was taken for each hind paw on each test, with the test sequence repeated at 5, 15, 30, 60, and 90 min after injection. The average of right and left hind-paw latency was defined as paw withdrawal latency (PWL).

*Hyperesthetic Rat Study*

Consistent with previous reports,<sup>7</sup> preliminary studies carried out in our laboratory‡ revealed that the maximum hyperesthesia occurred between 7 and 14 days after nerve ligation. Thus, each hyperesthetic animal was used only twice, at 7 and 11 days after nerve ligation, in these studies. Before drug injection, the hind paws were tested alternately three times, with 5-min intervals between the repeated testing of one paw as the baseline data. The left and right test sequence was carried out at 5, 15, 30, 60, and 90 min after injection.

In separate groups of rats, to verify that the analgesic effects of the several intrathecally administered agents were due to interaction at their respective receptors, the highest dose of each agonist was administered, followed at the appropriate time interval by the respective antagonist. Preliminary studies were carried out to assess the time of peak antagonist effects, and the doses used were selected on the basis of previous studies with intrathecal agonists.

‡ Yamamoto T, Yaksh TL: Unpublished data.

## DRUGS AND INJECTION

The agents administered intrathecally in this study were morphine sulfate (Merck), U50-488H (U50; Upjohn), (D-Pen2, D-Pen5)-enkephalin (DPDPE; Peninsula Laboratories); ST-91 (Boehringer Ingelheim), 5'-N-ethylcarboxamide-adenosine (NECA; Sigma), baclofen (CIBA) and muscimol (CIBA). Morphine, U50, DPDPE are  $\mu$ ,  $\kappa$ , and  $\delta$  agonists, respectively.<sup>7</sup> ST-91, NECA, muscimol, and baclofen are  $\alpha_2$ , adenosine, GABA-A and GABA-B agonists, respectively.<sup>8</sup>

For antagonism studies, the following agents were administered: morphine (30 nmol) followed at 30 min by naloxone HCl (5  $\mu$ mol/kg, intraperitoneally [ip]; Du Pont); DPDPE (155 nmol) followed at 10 min by naloxone (5  $\mu$ mol/kg, ip); ST-91 (120 nmol) followed at 30 min by atipamezole (1.2  $\mu$ mol/kg, ip; Farnos); NECA (1 nmol) followed at 10 min by caffeine (2  $\mu$ mol, intrathecally; Sigma); baclofen (5 nmol) preceded by 10 min by Phaclofen (120 nmol, intrathecally; RBI). These injection intervals were chosen as times representing peak effects of the agonist and antagonist at the time of testing.

## DATA ANALYSIS AND STATISTICS

The means  $\pm$  standard errors of the PWL were plotted. To define the magnitude of the hyperesthesia in a given rat, the difference score (difference in PWL) was computed by subtracting the latency of the control side (left side) from the latency of the ligated side (right side). Negative difference scores thus indicate a lower threshold on the ligated side, *i.e.*, hyperesthesia.

For the normal rat study and nonligated paw study, the percent maximum possible effect (% MPE) was calculated, where % MPE =  $\frac{[\text{postdrug response latency} - \text{predrug response latency}] + [\text{cut-off time (20 s)} - \text{predrug response latency}]}{\text{cut-off time (20 s)} - \text{predrug response latency}} \times 100$ . To obtain dose-response curve, the dose was plotted against the MPE. MPE of each paw was defined as the % MPE, which was the maximum during the first 30 min after drug injection. This was believed acceptable because of the similar baseline values in the nonligated paw of the lesioned animal and the normal paws in the nonlesioned animal. Dose-response curves were established with a least-squares linear regression analysis, and the values of doses that resulted in 50% MPE (MPE-ED<sub>50</sub>) and their 95% confidence intervals determined. The slopes of each regression line with the 95% confidence intervals were also calculated and used when testing for parallelism.<sup>9</sup>

To compare the analgesic effects of drugs in the ligated paw with those in the nonligated paw, the dose was plotted against the maximum PWL. The dose-response line in the ligated and nonligated paw were fitted using least-squares linear regression. Because of the pronounced and systematic differences in baseline response latencies, the

use of the % MPE was not deemed suitable. For this reason, a fixed response criteria, a PWL = 15 s, was chosen because it represents a change of approximately 3 standard deviations (SD) from baseline. The doses that resulted in that response latency (ED<sub>15s</sub>) were calculated along with their 95% confidence intervals. The slopes of each regression line and the 95% confidence intervals were also calculated.<sup>9</sup>

The paired and unpaired *t* test and one-way analysis of variance were carried out using a Dunnett test. *P* < 0.05 levels were considered significant.

## Results

### BASELINE DATA

In normal, nonsurgically treated animals, the average baseline PWL ( $\pm$  SD) was  $9.8 \pm 1.7$  s (*n* = 75). The mean baseline PWL ( $\pm$  SD) in the nonligated paw of the lesioned animal was  $10.6 \pm 2.0$  s (*n* = 108). The PWL of the nonligated paw, though modest, was significantly different from that of the nonsurgically treated animals (*t* = 2.714, degrees of freedom [df] = 182; *P* < 0.01). In the lesioned rat, the mean PWL ( $\pm$  SD) for the ligated paw ( $8.2 \pm 1.7$  s) was significantly less than that of either of the nonligated paws (*t* = 2.406, df = 107; *P* < 0.0001) or the response latency of the nonsurgically treated animals (*t* = 6.55, df = 182; *P* < 0.0001), with the average difference score ( $\pm$  SD) being  $-2.5 \pm 1.7$  s (*t* = -15.0, df = 107; *P* < 0.0001).

### GENERAL BEHAVIORAL EFFECTS OF INTRATHECAL AGENTS

In the normal rat study, the highest drug doses used were those in which the paw response was blocked (PWL = 20 s) or in which there was evidence of mild to moderate motor dysfunction. U50 (430 nmol), muscimol (22 nmol), baclofen (5 nmol), and NECA (1 nmol) produced motor dysfunction in a significant number of animals and represented the limiting factor in studying their effects. At maximally effective doses in normal or ligated rats, no motor effects were observed for morphine, DPDPE, or ST-91.

### TIME COURSE

The typical time course of PWL after intrathecal morphine or ST-91 injection in the normal animal and the hyperesthetic animal (ligated paw and nonligated paw) are illustrated in figure 1. These data show that administration of a just maximally effective dose of morphine (30 nmol) or ST-91 (40 nmol) resulted in a significant increase in the PWL in both normal and hyperesthetic animals. Morphine produced a longer-lasting elevation of PWL for the nonligated paw than for the normal rats' paw.

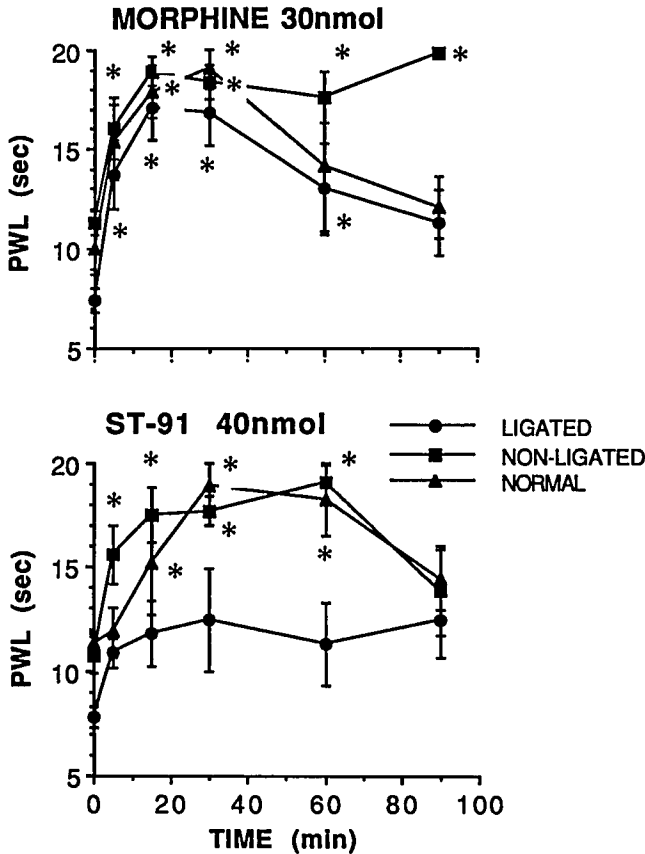


FIG. 1. Effects of intrathecal MOR (30 nmol) and ST91 (40 nmol) on the thermal nociceptive threshold. Ordinate: paw withdrawal latency (PWL). Abscissa: time in minutes after drug injection. Each line represents the mean  $\pm$  SEM of determinations made in three to six rats. \* $P < 0.05$  compared to time 0. Ligated = ligated paw of hyperesthetic rat study; Nonligated = nonligated paw of hyperesthetic rat study; normal = normal rat study.

#### INTRATHECAL AGONISTS IN THE NORMAL RAT

Log dose-response (% MPE) curves for the effects of morphine, U50, DPDPE, ST-91, NECA, baclofen, and muscimol on the PWL in the normal animal are shown in figure 2. Table 1 presents MPE-ED<sub>50</sub> values and their 95% confidence intervals. Morphine, DPDPE, ST-91, baclofen, and muscimol could produce an increase in the response latency in a dose-dependent manner, whereas U-50 and NECA did not increase the PWL in a dose-dependent manner at doses that did not produce prominent motor dysfunction. The order of the potency was: baclofen, ST-91, morphine, muscimol, DPDPE  $\gg$  U-50, NECA  $\geq$  0.

#### INTRATHECAL AGONISTS IN THE HYPERESTHETIC RAT

The slopes and MPE-ED<sub>50</sub> values of the log dose-response (% MPE) curve for the nonligated paw of the hy-

peresthetic rats are given in table 1. With intrathecal DPDPE, muscimol, and ST-91, the slopes and ED<sub>50</sub> values of the nonligated paw in the hyperesthetic animal were not different from that determined in the normal rat.

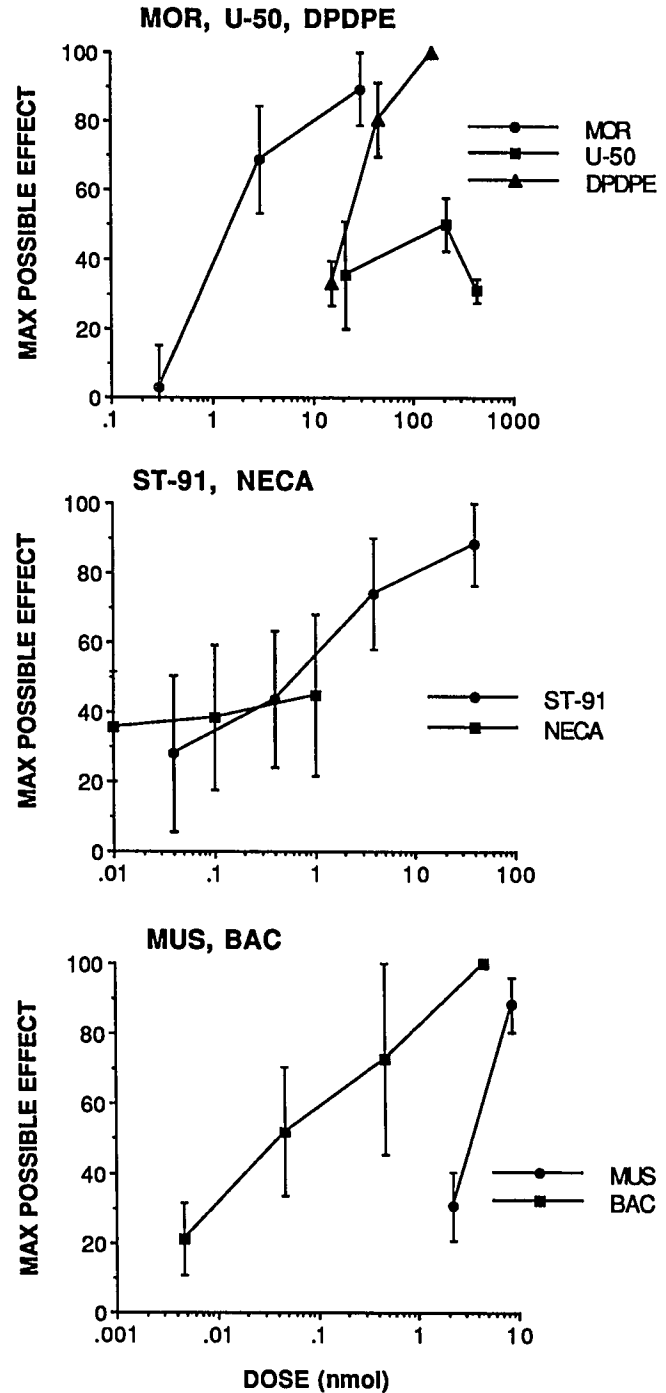


FIG. 2. Log dose-response curves for the effects of MOR, U50, DPDPE, ST91, NECA, MUS, and BAC on the thermal nociceptive threshold in the normal rat study. Ordinate: maximum (MAX) possible effect. Abscissa: log dose (nanomoles). Each point represents the mean  $\pm$  SEM of three or four rats.

TABLE 1. Dose-response Analysis: MPE-ED<sub>50</sub> and Slope with 95% Confidence Intervals

Drug	Normal Rat		Hyperesthesia Rat (Nonligated Paw)	
	MPE-ED <sub>50</sub> (nmol) (95% CI)	Slope (95% CI)	MPE-ED <sub>50</sub> (nmol) (95% CI)	Slope (95% CI)
Morphine	1.8 (0.6-5.4)	39.6 (18.9-60.2)	0.3* (0.09-0.9)	27.6 (17.1-38.2)
U50	>100	—	>100	—
DPDPE	23.7 (15.6-36.2)	67.7 (42.3-93.0)	22.9 (9.4-23.3)	70.1 (43.5-96.7)
ST91	0.4 (0.04-3.4)	19.9 (3.4-36.3)	0.6 (0.2-2.4)	19.7 (11.5-27.9)
Baclofen	0.05 (0.009-0.3)	25.7 (9.3-42.1)	0.09 (0.05-0.2)	71.7* (32.3-111)
Muscimol	3.5 (2.6-5.3)	96.0 (39.2-153)	4.4 (2.6-7.9)	72.8 (31.9-114)
NECA	>1	—	0.06 (0.01-0.3)	34.3 (7.8-60.9)

Shown are the MPE-ED<sub>50</sub> and slope with 95% confidence intervals of the %MPE observed after the intrathecal administration of receptor-preferring agonists on the hindpaw thermal withdrawal latency in the paws of normal rats and in the nonligated paws of rats with sciatic nerve partial ligation.

Dash indicates inactive.  
CI = confidence interval; DPDPE = (D-Pen2, D-Pen5)-enkephalin;  
NECA = 5'-N-ethylcarboxamide-adenosine.  
\* Significantly different from the normal rat (*P* < 0.05).

With morphine and NECA, there was a significant increase in activity in the nonligated (nonhyperesthetic) paw as compared to the normal rats' paw. As in the normal paw, intrathecally administered U-50 had no dose-dependent effect in the nonligated paw. The order of the activity in the nonligated paw was: NECA, baclofen, morphine, ST-91, muscimol, DPDPE > U-50 ≥ 0.

The systematic differences in baseline resulting from the ligation can yield misleading interpretations of the relative potencies of these agents in the animals with lesioned and nonlesioned nerves. Thus, in figure 3, drug doses were plotted against the maximum PWL observed in animals with ligated and nonligated sciatic nerves. Table 2 presents the slopes and the ED<sub>15s</sub> values for the ligated and the nonligated paw. With morphine, DPDPE, and muscimol, the dose-response curves were shifted to the right in a parallel fashion. With ST-91, the dose-response curve for the ligated paw showed a limited efficacy, with a plateau effect observed at doses that completely blocked the nonligated paw response. U-50 could produce no effect in either the ligated or nonligated paw. NECA, in contrast to its effect on the nonligated paw, had no effect on the response latency of the nerve ligated paw; rank ordering of activity in the hyperesthetic paw was: baclofen, morphine, muscimol, DPDPE > ST-91, NECA, U-50 ≥ 0. As emphasized in tables 1 and 2, these results resemble those observed with these drugs in the normal, nonlesioned animal.

Inspection of the dose-response data also yields the interesting observation that for all of the agents, with the exception of NECA and baclofen, all doses produced approximately equal increments in paw latency; *i.e.*, the slopes were essentially parallel. In contrast to morphine, DPDPE, ST-91, and muscimol, however, the two agents NECA and baclofen clearly abolished the hyperesthesia at the lowest dose that we used in this study (see latency of the ligated *vs.* nonligated paw in fig. 3). For these two

agents, at the lowest doses examined, the PWL for the hyperesthetic paw was preferentially elevated to the level of PWL of the nonligated paw (fig. 3).

#### ANTAGONIST STUDY

Figure 4 presents the time course of the reversal of the increased PWL latency by opioid and α<sub>2</sub> antagonists otherwise produced by the intrathecal administration of morphine and ST-91, respectively, in the hyperesthetic animal. Table 3 indicates the antagonistic effects of naloxone, atipamezole, caffeine, and Phaclofen against the action of the respective receptor agonist. Caffeine alone at the dose used did not antagonize the effect of NECA in this model.

#### Discussion

It has been shown that the application of loose unilateral sciatic nerve ligations will yield a pronounced time-dependent thermal<sup>4</sup> and mechanical hyperesthesia.<sup>10</sup> The average difference score observed in the present series of studies was approximately -2.5 s at 7-14 days after the application of loose sciatic ligatures, a value comparable with that previously reported.<sup>4</sup> Although a difference in score can reflect either hyperesthesia of the lesioned paw or an analgesia of the nonligated paw, examination of the data revealed that the ligated paw showed a highly significant decrease in response latency when compared to the nonlesioned animal or the contralateral nonligated paw. Thus, although the nonligated paw in operated animals revealed a modest increase in withdrawal latency, the lesion did in fact result in a hyperesthesia as compared to the normal, nonlesioned population.

Although the specific mechanisms underlying these prominent changes are not known, several lines of evidence emphasize that the peripheral nerve lesion may evoke a major reorganization of dorsal horn function.

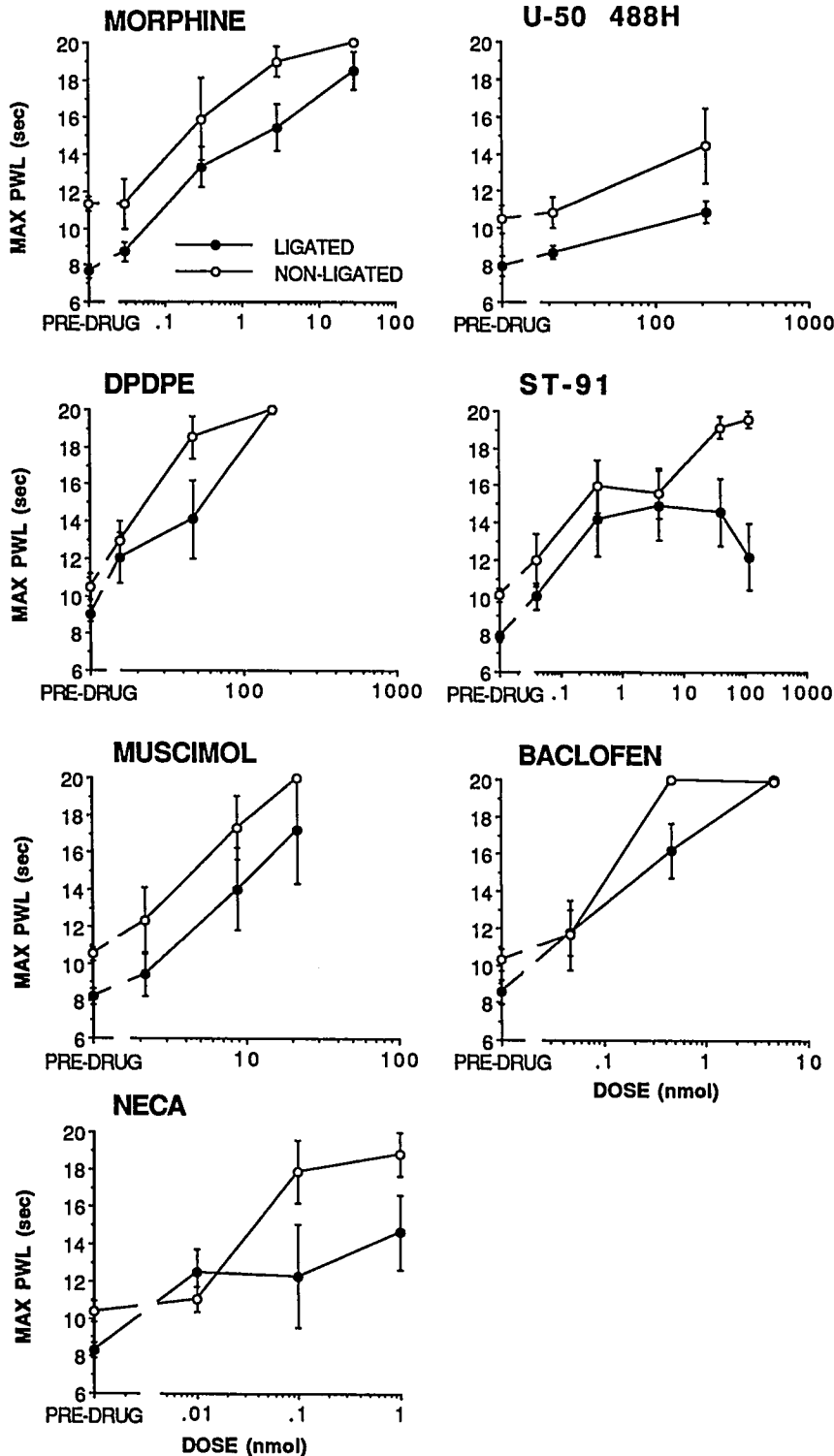


FIG. 3. Log dose-response curve for the effect of MOR, U50, DPDPE, ST91, NECA, MUS, and BAC on the thermal nociceptive threshold in the hyperesthetic rat study. Ordinate: maximum paw withdrawal latency (MAX PWL). Abscissa: log dose (nanomoles). Each point represents the mean  $\pm$  SEM of four to seven rats. Ligated = ligated paw of hyperesthetic rat study; nonligated = nonligated paw of hyperesthetic rat study.

Histologic examination of the nerve showed that after partial ligation, all of the large  $A\beta$  axons and a large percentage of the  $A\delta$  axons are damaged, but most of the C fibers appear anatomically intact.<sup>11</sup> Electrophysiologic studies revealed that ectopic discharges occur in the dam-

aged myelinated primary afferent nerves after the loose sciatic nerve ligations.<sup>12,13</sup> In the spinal cord, incomplete sciatic nerve ligations decreased the level of substance P and calcitonin gene-related peptide, presumably in the central terminals of small primary afferent nerves—ob-

TABLE 2. Dose-response Analysis: ED<sub>15</sub> and Slope with 95% Confidence Intervals

Drug	Normal Rat		Hyperesthesia Rat			
	ED <sub>15</sub> (nmol) (95% CI)	Slope (95% CI)	Ligated Paw		Nonligated Paw	
			ED <sub>15</sub> (nmol) (95% CI)	Slope (95% SI)	ED <sub>15</sub> (nmol) (95% CI)	Slope (95% CI)
MOR	2.2 (0.8-6.6)	4.0 (1.8-6.3)	2.0 (0.9-4.7)	3.1 (2.0-4.1)	0.2* (0.07-0.9)	2.8 (1.7-3.9)
U50	—	—	—	—	—	—
DPDPE	29.1 (18.7-45.2)	7.9 (4.5-11.2)	43.0 (25.2-73.5)	8.0 (3.5-12.5)	23.7 (13.8-40.9)	70 (3.8-10.2)
ST91	0.3 (0.03-2.5)	1.9 (0.4-3.4)	—	—	0.6 (0.2-2.3)	2.0 (1.2-2.8)
BAC	0.07 (0.01-0.4)	2.7 (0.81-4.5)	0.3 (0.1-0.6)	4.1 (2.5-5.8)	0.1 (0.06-0.2)	8.4† (3.8-13.0)
MUS	3.2 (2.0-5.1)	9.2 (3.6-14.8)	11.6 (5.0-26.7)	7.7 (2.0-13.5)	4.7† (2.6-8.6)	7.8 (3.1-12.4)
NECA	—	—	—	—	0.06 (0.02-0.2)	3.9 (1.7-6.1)

Shown are the ED<sub>15</sub> (the dose required to raise the response latency to 15 s) and slope with 95% confidence intervals for intrathecally administered receptor-preferring agonists in the paws of normal rats and in the normal and hyperesthetic paw of rats with unilateral partial

ligation of the sciatic nerve.

Dash indicates inactive.

\* Significantly different from the ligated paw ( $P < 0.005$ ).

† Significantly different from the ligated paw ( $P < 0.05$ ).

servations similar to those made after complete nerve section.<sup>14</sup> In addition to the anticipated changes in the central terminals of the axon, systematic examination of the dorsal

horn revealed that there were prominent anatomic signs consistent with transsynaptic changes in dorsal horn function. Thus, there is a time-dependent appearance of numerous dark-staining neurons in the dorsal horn, indicative of cell dysfunction. In this model, the incidence of neurons with signs of transsynaptic degeneration was increased in the lumbar dorsal horn on both the ligated and nonligated sides.<sup>15</sup> Thus, although the mechanisms of the hyperesthesia following nerve ligation are not known, the associated changes in dorsal horn morphology and chemistry suggest a significant reorganization of function. Of particular significance is that many of these changes evoked by unilateral nerve section suggest that the ipsilateral nerve insult may unexpectedly induce contralateral changes in spinal afferent processing.

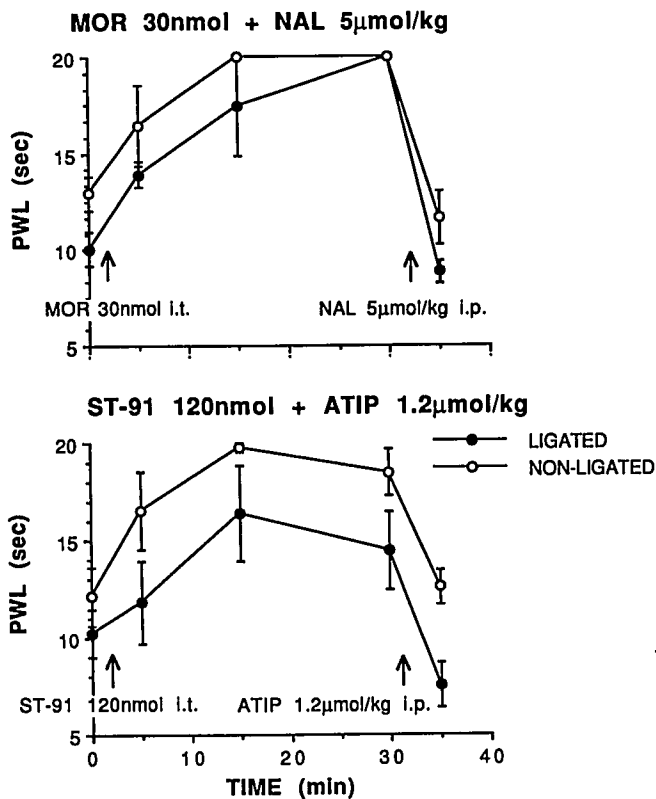


FIG. 4. Time course of paw withdrawal latency (PWL) of the MOR (30 nmol intrathecally) + NAL (5 µmol/kg intraperitoneally) study and ST91 (120 nmol intrathecally) + ATIP (1.2 µmol/kg intraperitoneally) study in the hyperesthetic rat. Ordinate: paw withdrawal latency (PWL). Abscissa: time (minutes). Each line represents the mean ± SEM of determinations made in four rats. Ligated = ligated paw of hyperesthetic rat study; nonligated = nonligated paw of hyperesthetic rat study.

#### NOCICEPTIVE MEASUREMENTS

The use of the plantar thermal stimulation model, as described by Hargreaves *et al.*,<sup>6</sup> was found to be clearly suitable as a model of assessing the antinociceptive action of spinally administered drugs. Two very important characteristics of this thermal test paradigm that distinguish it from the hot-plate test are 1) the ability to carry out differential assessment of changes in the thermal nociceptive threshold in either paw, and 2) the ability to assess response latencies with no handling or restraint, a particular advantage in a hyperesthesia model. Insofar as spinal drug action is concerned, in normal animals the order of the relative activity of the several agents examined was observed to be similar to that observed on other thermal stimulation devices, such as the 52.5° C hot-plate test.<sup>16-19</sup> The lack of effect of U50 (a κ agonist) or NECA (A1/A2 receptor types) is consistent with their mild effects observed even at high doses on the hot-plate endpoint.<sup>20,21</sup> Analysis of the data in this model appears amenable to both the use of normalization procedures (such as percent

TABLE 3. Effects of Antagonists on the Effects of Receptor-preferring Agonists on the Thermal Response Latency

	Post Intrathecal Drug Response (s)	
	Ligated Paw	Nonligated Paw
MOR	20 ± 0	20 ± 0
MOR + NAL	9 ± 1	12 ± 3
DPDPE	17 ± 3	20 ± 0
DPDPE + NAL	8 ± 1	11 ± 2
ST91	15 ± 4	19 ± 2
ST91 + ATIP	8 ± 2	13 ± 2
BAC	20 ± 0	19.9 ± 0.3
BAC + PHAC	12 ± 2	14 ± 5
NECA	13 ± 2	19 ± 2
NECA + CAF	14 ± 2	18 ± 2

Shown are effects of antagonists on the effects of intrathecally administered receptor-preferring agonists on the thermal response latency of the normal and hyperesthetic paw in rats with unilateral partial ligation of the sciatic nerve.

Each value represents the mean ± SD of four or five rats. Data are expressed with PWL.

of baseline or % MPE), as well as the selection of a fixed response latency. Where there are systematic changes in baseline, as occurs in the nonligated and ligated paw, such comparisons may be misleading and obscure differences in drug effect. For that reason, in the present study, we elected to also carry out comparisons using a fixed threshold (PWL = 15 s) which, based on the baseline variance, corresponds to a statistically significant elevation.

A problem that can influence all thermal tests is the effect of cutaneous temperature. Data in the tail-flick test has suggested that reductions in skin temperature may increase response latencies and *vice versa*.<sup>22</sup> To address this issue, we have prepared the device originally outlined by Hargreaves and colleagues<sup>6</sup> and in addition have placed a small, thermostatically controlled heater blower unit in the case underlying the glass plate of the testing area, which maintains plate temperature at 30° C. Although we have not systematically measured paw temperature, this addition would clearly diminish the influence of differential changes in paw temperature due to systematic alterations in local plantar vasomotor tone due either to the lesion or to the effects of intrathecal or systemically administered agents.

Another possible problem is that the glass plate used in this thermal-stimulation model acts as a heat sink. Hirata *et al.*<sup>23</sup> observed that human subjects consistently withdraw at approximately the same temperature whether the skin was in contact with the glass or not, but that their withdrawal times were more rapid when the skin surface was not in contact with the glass.<sup>23,24</sup> In this study, we positioned the thermal stimulator so that only the skin surface was in contact with the glass plate. Moreover, in our study,

we used a much smaller aperture for the radiant heat source (4 mm in diameter) than did Hargreaves *et al.* (5 × 10 mm).<sup>6</sup> This change may be helpful to avoid an unintentional stimulation of the skin surface that was not in contact with the glass. Thus, we believe that the measurement in this study was reliable and that the faster withdrawal latency in the ligated paw was because of a true hyperesthesia, not simply because the skin surface was reaching a noxious temperature more rapidly.

#### EFFECTS OF SPINAL AGENTS IN THE HYPERESTHETIC RAT

In the present study, spinally administered receptor selective agents increased the hind-paw withdrawal latency for both the normal and ligated/nonligated paws. The results, however, were not as straightforward as might be anticipated. Several points should be emphasized.

First, a comparison between the effects of drugs on the response latency of normal rats and the nonligated paw of the lesioned animal using MPE-ED<sub>50</sub> or the ED<sub>15s</sub> revealed no differences for DPDPE, ST-91, baclofen, or muscimol but a significant *increase* in activity of morphine and NECA in the nonlesioned paw. Morphine produced a longer-lasting elevation of PWL for the nonlesioned paw than for the normal rats' paw. Because it was anticipated that the nonlesioned paw would be the control for the lesioned side, these differences from the nonlesioned animals were particularly surprising. Given that there was a little difference between predrug response latencies of contralateral side paws and that of normal rats' paw, this differential sensitivity cannot be attributed to different baselines. This augmented sensitivity in the contralateral side of the lesioned animal suggests that the contralateral changes in morphology and biochemistry noted above may have particular relevance to understanding the mechanisms of hyperesthesia.

Second, considering the lesioned rats, the intrathecal dose-response curves of the ligated paw were shifted to the right. For morphine, DPDPE, and muscimol, this appeared to occur in a parallel manner. This parallelism may be interpreted to indicate that the hyperesthetic paw of the animal is either more or less sensitive than the nonligated paw to the antinociceptive actions of the agents examined. Thus, it would be anticipated that starting at a lower baseline would indeed necessitate an increase in dose to reach any given response criteria. (It is important that plotting these data using the percent change from baseline produces precisely overlapping dose-response curves and obscures this difference). ST-91 did present a clear plateau effect with the ligated paw. Given recent work from our laboratory that ST-91 is a partial agonist when examined in the presence of elevated thermal stim-



uli,§ we believe this result is consistent with the hypothesis that the nerve ligation results in changes that are modeled by increased gain of the input generated by a given thermal stimulus. In our recent studies, we indeed observed that the  $\alpha_2$  agonist dexmedetomidine could yield a maximum elevation of the PWL of the lesioned paw.¶

NECA and baclofen abolished the hyperesthetic state, but NECA failed to produce a significant antinociception in the ligated paw and normal paw. Previous studies with intrathecal baclofen and NECA have shown them to have a mild effect on the thermal nociceptive threshold, but the prominent increases were reliably obtained only at doses that appeared to produce motor dysfunction.<sup>16,21</sup> The selective effects observed in the present study on the thermal hyperesthesia of the nerve ligated paw were observed at doses that were considerably less than those that influence motor activity. These results thus further emphasize that the pharmacology of the lesion-evoked hyperesthesia can be differentiated from the pharmacology of the normal paw. Sosnowski and Yaksh reported that NECA could inhibit the allodynia induced by strychnine.<sup>25</sup> Hwang and Wilcox reported that baclofen blocked substance P spinal activity.<sup>26</sup> These observations suggest that the hyperesthetic state following sciatic nerve loose ligation had some similarities to the allodynia induced by intrathecal strychnine or substance P.

#### NEUROGENIC PAIN AND OPIOID SENSITIVITY

The continued effects of these receptor agonists in the nerve ligation model, suggesting that the changes in sensory processing retain an opioid sensitivity, might not be totally unexpected. As indicated, after ligation, unmyelinated afferent profiles remain, and these probably represent the principle pathways by which such input arrives in the spinal dorsal horn. The present observation showing retention of activity of opioid and  $\alpha_2$  agonists is consistent with the continued presence of pre- and postsynaptic receptors for these respective agents that modify this afferent-evoked activation of higher-order spinal projection neurons.<sup>8,27</sup> The retention of activity of these agents in the partial ligation model, where C fibers are preserved,<sup>11</sup> is in contrast to the loss of effect of morphine on the spontaneous single-unit activity observed after dorsal rhizotomy, where spontaneous activity is independent of afferent input.<sup>28</sup> Thus, the present data, showing the parallel shift in the curves, suggest that there has been an increased drive of the systems that process pain information or a change in the coupling number of the opioid receptor. Whether the facilitation of the response is due to an exaggerated presynaptic release of excitatory neu-

rotransmitter (as suggested by the up-regulation of messenger RNA or levels of some neurotransmitters, but not others) or an exaggerated postsynaptic response because of a loss of intrinsic inhibition (as suggested by the fallout of interneurons) is not known. In either case, the substrate would retain a predicted sensitivity to the modulatory substrates that control the spinal output of information otherwise evoked by C-fiber input. The rightward shift in the dose-response curve would thus be anticipated, since the opioid/ $\alpha_2$  receptor occupancy necessary to reduce such elevated drive would be increased.<sup>29</sup>

#### ORIGIN OF HYPERESTHESIA

If there is an exaggerated drive in the dorsal horn that occurs because of either trophic changes or the loss of specific modulatory systems, it will be important to define the nature of the receptor systems responsible for the facilitated response. As noted, little evidence was found with the spinal receptor systems examined that there was a selective effect upon the hyperesthesia. In previous studies, there is evidence that the exaggerated sensitivity to low-threshold tactile input was related to the loss of glycine or GABA-A input,<sup>30</sup> consistent with the presence of GABA and glycine neurons in the substantia gelatinosa.<sup>31</sup> Consistent with the role of large, low-threshold afferent nerves, this input was relatively insensitive to opioids and  $\alpha_2$  agonists. It is important that the hyperesthesia is completely reversed by N-methyl-D-aspartate antagonists<sup>30</sup> and by adenosine agonists that block the release of glutamate.<sup>25</sup> Continuing studies with the nerve injury models have also recently shown, in contrast to the receptor systems involved in the present study, that the spinal N-methyl-D-aspartate receptor appears to modulate specifically the hyperesthetic phases observed following partial nerve ligation.\*\* It is important that in the present study, NECA and baclofen, unlike any of the other agonists, yielded a reduction in the hyperesthesia at the lowest doses that failed to alter the normal paw latencies. Therefore, it appears possible that these two separate spinal models of aberrant sensory processing, one reflecting an allodynia mediated by low-threshold myelinated and the other a hyperesthesia mediated by high-threshold input, respectively, have some aspects of dorsal horn pharmacology in common.

In conclusion, the present studies demonstrated that the thermal hyperesthesia evoked by a unilateral peripheral constriction injury of the sciatic nerve displays a significant sensitivity to  $\mu$ ,  $\delta$ , and  $\alpha_2$  agents. In addition, however, the ability to selectively diminish the hyperesthesia by adenosine and GABA agents suggests that the hyperesthetic state reflects more than a simple change in

§ Saeki S, Yaksh TL: Manuscript in preparation.

¶ Yamamoto T, Yaksh TL: Unpublished data.

\*\* Yamamoto T, Yaksh TL: Unpublished data.

the gain of the spinal systems that process nociceptive information in the unlesioned animal. From a clinical perspective, these studies reveal that possibility that certain neurogenic pain may display useful opioid sensitivity.

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